Remarks

Favorable consideration of this application is respectfully requested in view of the foregoing amendment and the following remarks.

Claims 1-49 are pending in the application. Claims 1-49 have been rejected. Claims 1, 17, 23, 35 and 47 have been amended. No new matter has been added.

Claims 1-49 have been rejected under 35 U.S.C. §112, second paragraph, as being indefinite. In particular, the Examiner has rejected the phrases "equal protein pools" (Claims 1, 23, 35 and 47), "inverse labeling pattern" (Claims 1, 23, 35 and 47), "during labeling (claim 23), and "proteolyzing...with isotopically labeled water"(Claims 23 and 35). Each ground for the §112, second paragraph, rejection is addressed below.

With respect to the phrase "equal protein pools" the Examiner stated:

> In claim 1, step (a), the recitation of "equal protein pools" is indefinite because it is not clear how protein pools are "equal". It is not clear how a reference sample pool is "equal" to an experimental sample pool.

Applicants respectfully disagree with the Examiner's conclusion and assert that the phrase "equal protein pool" is definite. It is noted that step (a) of Claims 1, 23, 35 and 47 as amended recites "providing two equal protein pools from a reference sample and two equal pools from an experimental sample." Support for the amendment to Claims 1, 23, 35 and 47 is found throughout the specification, e.g., on pages 11, 12, 18 (second paragraph) and 21 (Example 1). "Two equal protein pools" provided from a reference sample or an experimental sample means that the two protein pools are the same with respect to a specified amount taken from the reference sample or the experimental sample, e.g., a specified amount derived from the same number of cells or contained the same amount of total proteins, which is well known in the art in performing comparative proteomics. An example of the meaning of "two equal protein pools" is also illustrated in the specification on page 21 (Example 1) wherein it indicates that two identical aliquots containing 10 pmol each of the unchanged components are taken from the control pool and are also taken from the treated pool. That the term "equal" with respect to two protein pools is a term well known in the art and refers to two protein pools having the same specified amount is also illustrated, e.g., in Diamond et al., Molecular Endocrinology, Vol. 13 (2): 228-238 (1999) (Diamond et al.), a copy of which is enclosed with this amendment. Therein, Diamond et al., in the Figure 2 legend, states that when running a gel, "Lanes were loaded with equal protein (100ug)....[emphasis added with underline]" Diamond et al. in the Figure 2 legend subsequently states "To demonstrate equal protein loading, the blot was stripped and probed with a mouse monoclonal antiactin antibody." [emphasis added with underline] [See also

Diamond et al., page 230, right middle: "The blot was reprobed with a monoclonal antiactin antibody to demonstrate that all lanes were loaded with equal amounts of cell lysate (Fig. 2C)"]. Thus, Diamond et al. makes clear that the term "equal' when used in the phrase "equal protein loading" refers to loading the same amount of protein in each lane of the gel. Accordingly, one skilled in the art armed with knowledge in the art and specification would understand that the meaning of "equal" in the phrase "two equal protein pools" refers to two protein pools of the same specified amount.

It is also noted that in practice one skilled in the art would also understand that the two protein pools may not always be exactly the same with respect to a specified quantity (i.e., at a ratio that is not equal to one). To account for any small difference in the amounts of the two protein pools, the signals may be normalized before comparative analysis or a statistical analysis can be performed on the ratios to identify the statistically significant differential changes.

With respect to the phrase "inverse labeling pattern" the Examiner states on page 3 of the outstanding Action:

> In claim 1, step(f), the recitation of "inverse labeling pattern" is indefinite because the scope of the definition of the term "inverse" is not clear in both Applicants' specification and Applicants' clarifying remarks. Applicants argue that the term "inverse labeling pattern" is broadly defined on page 11 of the specification meaning "a qualitative mass shift or an isotope peak intensity ratio reversal" (See Applicants' Reply at p.4, second paragraph, lines 2-4) (emphasis added). However, Applicants also acknowledge that the term " inverse" has a more limited definition meaning "a reversal in the signal intensity ratio" (see Applicants' Reply at sentence bridging pp. 4-5). Both Applicants' specification and Applicants' clarifying remarks do not appear to resolve how a "qualitative mass shift" amounts to the creation of an "inverse labeling pattern" or how the definition of the term "inverse", dictionary or otherwise, applies to the concept of a qualitative mass shift."

Applicants respectfully disagree with the Examiner's conclusion and submit that the phrase "inverse labeling pattern" is definite. As asserted in the previous response, the phrase "inverse labeling pattern" is defined on page 11 of the application as "a qualitative mass shift or an isotope peak intensity ratio reversal". While Applicants stated in the paragraph bridging pages 4-5 that the term "inverse" with respect to labeling pattern is a reversal in the signal intensities of the light and heavy isotope" this statement is not a more limited definition of "inverse labeling pattern" as asserted by the Examiner. The term "qualitative mass shift" is a sub type of "isotope peak intensity ratio reversal" that occurs in limited situations as is explained below.

In situations in which the signal from an isotopic peak is weak and is not detected (i.e., below the detection limit), the stronger isotopic peak may be the only one detected from the isotopic pair of the peptide. As a result, the isotopic peak intensity ratio reversal, appears as a qualitative mass shift, i.e., the detection of two singlets, one from each mixture sample, one at the light isotope labeled mass and the other at the heavy isotope labeled mass. For example, extreme changes in protein expression or protein covalent modification can cause only one peak (heavy or light labeled) of a pair to be detected in an analysis, i.e., the lesser abundant peak in an isotopic pair may be below detection limit and not be detected. After subtractive comparison between two analyses (in which the isotope labeling is reversed between the two samples in the second experiment) (see Figure 1, left side of pattern) these peaks, which appear as a single peak in a given analysis have a distinct labeling mass shift pattern with the mass difference equal to the mass difference between the heavy and light labeled peptide. Accordingly, if a protein is differentially expressed and a weak isotopic peak is below the detection limit, a reversal in the signal intensity will show up in the pattern as two singlets at different masses (the light and heavy isotope) between the two experiments, i.e., a qualitative mass shift. It is further noted that chemical background or protein/peptides that didn't incorporate labels, would be detected as singlets and do not change in mass between the inverse labeling experiments and would cancel each other out when performing subtractive comparison. Accordingly, one skilled in the art reviewing the specification and Figure 1 would understand that a "qualitative mass shift" is a sub type of isotopic peak intensity ratio reversal, and would also understand the meaning of "inverse labeling pattern" as "an isotopic peak intensity ratio reversal" including the extreme situations where two singlets are detected with the mass difference equal to the mass difference between the heavy and light labeled peptide or "a qualitative mass shift".

With respect to the Examiner's contention that the recitation of "during labeling" lacks antecedent basis in Claim 23, to facilitate prosecution while not necessarily agreeing with the ground for this rejection, the phrase "proteolyzing each protein pool during labeling with isotopically labeled water" in independent Claim 23 has been replaced with the phrase "proteolyzing each protein pool in the presence of isotopically labeled water." Support for the language in this phrase can be found in the specification, e.g., page 14, lines 18-21, page 15, lines 6-9, page 16, third full paragraph, lines 1-3, and page 21, Example 1.

With respect to the Examiner's contention that the recitation of "proteolyzing... with isotopically labeled water" in Claim 23 is indefinite, because it is unclear how, by itself, isotopic water is capable of proteolysis, it is reiterated that Claim 23 (b) as amended recites "proteolyzing each protein pool in the presence of isotopically labeled water." The meaning of the words "proteolyzing each protein pool..." is clear from the specification on page 15, lines 1-4, which indicates that proteolysis of the proteins in the pool is effected using various

proteolytic agents such as proteases, e.g., trypsin, chemicals, e.g., cyanogens bromide; or dilute acids, e.g., hydrogen chloride. That proteolysis of protein pools can be effected in the presence of isotopically labeled water is explained in the specification on page 16, lines 18-26, which states

> "....protein pools of the reference and experimental samples are proteolyzed using trypsin prior to or at the same time of labeling with ^{18}O- and ^{16}O-water. One ^{18}O-atom and one ^{16}O-atom is incorporated into the newly formed carboxy terminus as a consequence of hydrolysis during proteolysis. An additional ^{18}O and ^{16}O may be incorporated into the terminal carboxy group through a mechanism of protease-catalyzed exchange as described, e.g., in Rose et al., 1988, supra. Thus, following digestion by trypsin all of the resulting peptides except for C-terminal peptides that lack Lys or Arg at the C-terminus are labeled with either one or two ^{18}O-and ^{16}O-atoms at the C-terminus (mostly two if enough time is allowed for exchange)."

Accordingly, one skilled in the art reading this disclosure in the specification would understand the meaning of the phrase "proteolyzing each protein pool in the presence of isotopically labeled water."

In view of the above, withdrawal of the rejection of Claims 1-49 under 35 U.S.C. §112, second paragraph, is respectfully requested.

Before addressing the other outstanding rejections, it is noted that Claim 17 has been amended to correct a minor typographical error. Specifically, the recitation of "^{14}C" in Claim 17 has been replaced by "^{13}C." Support for this amendment is found in the specification on page 10.

Claims 1-12, 14, 16-19, 21-22 and 47-49 have been rejected under 35 U.S.C. §102(e) as being anticipated by U.S. Patent No. 6,391,649 (Chait et al.). In particular, the Examiner stated:

> "Chait et al. describe a method for identifying a differentially expressed protein in two different samples (see Abstract, "comparing the levels of cellular components, such as proteins, present in samples which differ in some respect from each other") comprising the steps of: providing two equal protein pools (see col. 11, lines 56-57, "high abundance proteins derived from two pools") from each of a reference sample (see col. 12, lines 11-12, "expressing population is designated 'CLN2^{+}'") and an experimental sample (see col. 12, lines 12-13, "non-expressing population is designated 'cln2^{-}'"), labeling the protein pools with a substantially chemically identical isotopically different protein labeling reagent for proteins, wherein one pool from each of the reference and experimental pools is labeled with an isotopically heavy protein labeling reagent to provide an isotopically heavy-

labeled reference pool and an isotopically heavy-labeled experimental pool, and wherein the remaining reference and experimental pools are labeled with an isotopically light protein labeling reagent to provide an isotopically light-labeled reference pool and an isotopically light-labeled experimental pool (see col. 12, lines 14-18), combining the isotopically light labeled reference pool with the isotopically heavy-labeled experimental pool to provide a first protein mixture (see col. 12, lines 16-18, "A second combined sample contained 1 mL of unlabeled (^{14}N) extract of CLN2^{+} plus 1 mL of ^{15}N-labeled extract of clin2^{-}"), combining the isotopically heavy-labeled reference pool with the isotopically light-labeled experimental pool to provide a second protein mixture (see col. 12, lines 14-16, "A first combined sample contained 1 mL of unlabeled (^{14}N) extract of cln2^{-} plus 1 mL of ^{15}N-labeled extract of CLN2^{+}"), detecting the labeled proteins from each of the two mixtures (see col. 13, lines 14-15, "Mass spectrum measurements were obtained"), comparing the labeling pattern (see col. 14, lines 1.-3, "comparing the sum of the intensities of the isotopically resolved components of the unlabeled peptide with the corresponding sum from the ^{15}N peptide"), wherein an inverse labeling pattern is indicative of the differentially expressed protein (see Table IV)."

Applicants traverse this rejection and respectfully submit that Claims 1-12, 14, 16-19, 21-22 and 47-49 are not anticipated by Chait et al. Before addressing the Chait et al. reference, a brief summary of the presently claimed invention is stated below.

The present invention as defined in amended independent Claim 1 is directed to a novel method for identifying differentially expressed proteins or differentially modified proteins between two protein samples (e.g., a control vs. a disease or a control vs. a drug treated) that applies to all quantitative proteomics approaches based on the use of isotope labeling and mass spectrometry. In this method two equal protein pools are provided from a reference sample and two equal protein pools are provided from an experimental sample (step a). The protein pools are labeled with a protein labeling agent which is substantially chemically identical except that it is distinguished in mass by incorporating either a heavy or light isotope (step b). One pool from each of the reference and experimental samples is labeled with a light isotope and the remaining pools are labeled with a heavy isotope. To provide the first protein mixture for use in one of the two mass spectrometry experiments, a reference pool labeled with light isotope is combined with an experimental pool labeled with heavy isotope (step c). To provide the second protein mixture for use in the other mass spectrometry experiment, a reference pool labeled with heavy isotope is combined with an experimental pool labeled with light isotope (step d). Accordingly, the labeling of the reference and experimental pools of the first protein mixture is reversed in the reference and experimental pools of the second protein mixture. The labeled proteins from the two protein mixtures are detected (step e) and the labeling pattern obtained for the labeled proteins in the first and second mixtures are compared (step f). An

inverse labeling pattern of a protein in the second mixture compared with the labeling pattern of the protein in the first mixture is indicative of the differentially expressed protein in the two different samples (step f). Accordingly, in the presently claimed invention, rather than individually analyze each data set by pairing up the isotope pairs for each peptide and calculating the intensity ratios for every peptide and using the second data set for confirmation as is done with prior art methods (as discussed below with respect to Chait et al.), two data sets are compared, i.e., peaks are aligned between the two data sets rather than paired between each isotopic pair in each data set. Peaks of about similar relative intensity (normalized to a set of abundant peaks) are ignored. They include peaks from proteins of no significant change in expression or modification. Signals of interest, i.e., from proteins of differential expression or differential modifications can be unambiguously identified by viewing the inverse labeling pattern obtained for a peptide between the two data sets (see Figure 1), including singlet signals (from extreme differential expression or qualitative modification changes). Accordingly, with a protein that is differentially expressed or differentially modified, a reversal in the signal intensities of the light isotope and heavy isotope (the isotope pairs for each peptide) will be observed when comparing the two patterns obtained from the two protein mixtures. The presently claimed invention eliminates the need of analyzing the individual data set, i.e., pairing up isotope peaks for each peptide and then calculating peak intensity ratios for each peptide, which is a challenging task when dealing with a complex sample.

Chait et al. is directed to a method for comparing the levels of proteins in samples which differ in some respect from each other using mass spectroscopy and isotopic labeling. While Chait et al. in Example 2 performed two experiments, each experiment having two pools wherein the labeling of the two pools was reversed in the second experiment, the second experiment was performed for a very different purpose. The second experiment was carried out as part of the method validation, to rule out that any change in isotopic ratios was caused by the isotopic enrichment itself. In this regard, the Examiner's attention is directed to Chait et al., col. 8, lines 61-67, which states

> "To ensure that the change in the ratios is not caused by the isotopic enrichment itself, the process of Fig 3 is preferably repeated with the other cell pool being isotopically enriched, i.e., if in the first run the treated cell pool is isotopically enriched, as in FIG 3, then in the second run, the control cell pool would be isotopically enriched."

Accordingly, the purpose of performing the second experiment in Example 2 was to rule out that isotope enrichment (growing cell in heavy medium) was a source of ratio change. As also indicated in Chait et al., Example 2, col. 13, lines 64-67 and col. 14, lines 1-3, the intensity ratio of each protein in the two experiments was separately calculated and the results listed in Table IV (incorrectly labeled as Table III). Chait et al., then concluded (col. 14, line 63-67),

> "The normalized intensity ratios from the cln- (^{15}N)/CLN+ (^{14}N) (Column 6) cell pools were found to be in agreement with those from cln2- (^{14}N)/CLN+ (^{15}N) (Column 5) pools to within the statistical uncertainty of the measurement, as shown in Table III (IV), above."

Accordingly, Chait et al. calculated the intensity ratios of the pairs of peaks for each peptide in each data set produced from the two experiments and performed the second experiment to confirm the results of the first experiment to ensure that there was no artifact caused by isotope enrichment itself. Indeed, the Examiner has also acknowledged that the purpose of the second experiment in Chait was "to ensure that any change in isotopic ratios is not caused by the isotopic enrichment itself (see col. 8, lines 61-64)" (see outstanding Office Action on page 8, lines 1-2 and page 9, last two lines).

As evidenced in Table IV, the only way Chait et al. show that there was a differentially expressed protein, i.e., a protein whose abundance differed in a statistically significant manner from the other proteins, was to compare the intensity ratios of each of the proteins in the first experiment (see Table IV, incorrectly labeled as Table III, the intensity ratio of 0.58 for Triosephosphate isomerase versus the intensity ratio of other proteins in the first experiment). Chait et al. does not take the labeling pattern of the first experiment and compare it with the labeling pattern of the second experiment nor does Chait et al. indicate that an inverse labeling pattern, a reversal in the signal intensity ratio of the isotopic pairs of a peptide between the first experiment and the second experiment, is indicative of a differentially expressed protein as recited in step (f) of amended independent Claim 1. Since Chait et al. do not identically describe each and every element of amended independent Claim 1, Chait et al. fail to anticipate Claim 1.

In view of the above, withdrawal of the rejection of Claims 1-12, 14, 16-19, 21-22 and 47-49 under 35 U.S.C. §102(e) is respectfully requested.

Claims 23-28, 30, 32-40, 42 and 44-46 have been rejected under 35 U.S.C. §103(a) as being unpatentable over Schnolzer et al., Electrophoresis, vol. 17, pp. 945-953 (1996) (Schnolzer et al.) in view of Chait et al. In particular, the Examiner stated:

> "Schnolzer et al. teach a method of identifying proteins in a sample comprising the steps of: providing two equal protein pools (see p.950, col. 2, first full paragraph, line 2), proteolyzing each protein pool with isotopically labeled water (see p. 950, col.,2, first fully paragraph, line 3), combining the protein pools (see p. 950, col. 2, first full paragraph, line 2), detecting the labeled peptides (see p. 950, col. 2, first full paragraph, lines 15-17), and comparing the labeling pattern (see p. 950, col. 2, first full paragraph lines 6-8). Schnolzer et al. do not teach a method for identifying differentially expressed protein in two different protein samples."

The Examiner then reiterated his remarks regarding Chait et al. which were made to support the §102(e) rejection and concluded:

> "Therefore, it would have been obvious for a person of ordinary skill in the art to perform the method of identifying proteins in a sample, as taught by Schnolzer et al., to identifying differentially expressed protein in two different proteins samples because Chait et al. discovered a method for analyzing post-translational modifications (see col. 4, lines 11-14) to analyze the effects of environmental stimuli (e.g., drugs, hormones, etc.) in two or more biological samples (see col.3, lines 47-60) in order to gain insight into mechanisms of drug action, viral infection, etc. (see col.1, lines 28-54). In addition, Chait et al., discovered a method to ensure that any change in isotopic ratios is not caused by the isotopic enrichment itself (see col. 8, lines 61-64).
>
> With respect to claim 35, step (c), Schnolzer et al. teach a method wherein "peptide products continue to interact with these proteases and undergo repeated binding/hydrolysis cycles, resulting in complete equilibrium of both oxygens in the carboxy terminus of the fragment with oxygen from solvent water" (see Abstract)."

Applicants traverse this rejection and respectfully submit that the combination of Schnolzer et al. and Chait et al. does not make obvious Claims 23-28, 30, 32-40, 42 and 44-46 for the reasons stated below.

Schnolzer et al. is directed to a method of digesting proteins in enriched $H_2{}^{18}O$ for use in identifying proteins. While Schnolzer et al. indicate on p. 950, second full paragraph, that a protein sample is divided into two equal parts, and the parts are combined, Schnolzer et al. do not teach or specifically suggest a method for identifying differentially expressed proteins by performing a second experiment with a different protein sample which is also divided into two equal parts and combined, and wherein the label in the first experiment is reversed from the second experiment as set forth in steps (a-d) of Claim 23. Further, Schnolzer et al. do not teach or specifically suggest comparing the labeling pattern obtained for the labeled peptides in the first and second experiments (i.e., protein mixture in present claim 23), wherein an inverse labeling pattern of a peptide detected between the first and second experiments, is indicative of the differentially expressed protein from which the peptide originated.

As stated above in addressing the §102(e) rejection, Chait et al. fail to teach or specifically suggest comparing the labeling patterns obtained from the two experiments, nor does Chait et al. indicate that an inverse labeling pattern, a reversal in the signal intensity ratio of the isotopic pairs of a peptide between the first experiment and the second experiment, is indicative of a differentially expressed protein from which the peptide originated. Thus, Schnolzer et al. fail to remedy the deficiency present in Chait et al. Accordingly, the combination of Schnolzer et al. and Chait et al. does not make obvious Claims 23-28, 30, 32-40, 42 and 44-46.

In view of the above, withdrawal of the rejection of Claims 23-28, 30, 32-40, 42 and 44-46 under 35 U.S.C. §103(a) is respectfully requested.

Claims 13, 15 and 20 have been rejected under 35 U.S.C. §103(a) as being unpatentable over U.S. Patent No. 6,670,194 (Aebersold et al.) in view of Chait et al. In particular, the Examiner stated:

> "Aebersold et al. teach a method of identifying proteins in a sample comprising the steps of: providing two equal protein pools (see col. 5, lines 61-66), labeling each protein pool with an isotopically different labeling reagent (see col. 5, lines 61-66), combining the protein pools (see col. 6, lines 2-3), detecting the labeled peptides (see col. 6, line 9), and comparing the labeling pattern (see col. 6, line 13). With respect to claims 13 and 15, Aebersold et al. teach an affinity chromatographic fractionation of proteins (see e.g. col. 17, line 49, "panning") prior to step (a). With respect to claim 20, Aebersold et al. describe a labeling reagent containing an affinity tag (see Abstract)."
>
> "Aebersold et al. do not provide two protein pools from each of a reference and an experimental sample."

With respect to Chait et al., the Examiner reiterated his assertions made in the §102(e) rejection and then stated:

> "Therefore, it would have been obvious for a person of ordinary skill in the art to perform the method of identifying proteins in a sample, as taught by Aebersold et al., by providing two protein pools from each of a reference and an experimental sample because Chait et al. discovered that differentially expressed proteins can be analyzed for post-translational modifications (see col. 4, lines 11-14) to analyze the effects of environmental stimuli (e.g. drugs, hormones, etc.) on two or more biological samples (see col. 3, lines 47-60) in order to gain insight into mechanisms of drug action, viral infection, etc. (see col. 1, lines 28-54). In addition, Chait et al. discovered a method to ensure that any change in isotopic ratios is not caused by the isotopic enrichment itself (see col. 8, lines 61-64)."

Applicants traverse this rejection and respectfully submit that the combination of Aebersold et al. and Chait et al. does not make obvious Claims 13, 15 and 20 for the reasons stated below.

Aebersold et al. is directed to a method for quantitating proteins in one or more biological samples containing protein mixtures utilizing chemically identical, affinity tagged and differentially isotopically labeled reagents. In particular, Aebersold et al. teach the steps of providing a protein pool from two different samples, labeling the protein pools with an isotopically different labeling agent, combining the two labeled pools, enzymatically digesting the proteins to generate peptides, and detecting the labeled peptides. Aebersold et al., however, do not teach or specifically suggest

comparing the labeling patterns obtained from the two different pools as is asserted by the Examiner. Instead, Aebersold et al. indicate in col. 6, lines 12-15 that "The relative amounts of a given protein in each sample is determined by comparing relative abundance of the ions generated from any differentially labeled peptides originating from the protein." Accordingly, from the one experiment involving two protein pools labeled with a differentially isotopically labeled agent, Aebersold et al., as is the case in Chait et al., would have to calculate the isotopic peak intensity ratio (between the light isotope labeled peak and the heavy isotope labeled peak) for each peptide. The only way Aebersold would be able to show that there was a differentially expressed protein, i.e., a protein whose abundance differed in a significant manner from the other proteins was to compare the intensity ratios (calculated from the isotopic pair obtained for each peptide) of all the peptides in the one experiment to identify the differentially expressed proteins. As acknowledged by the Examiner, Aebersold et al. does not teach or specifically suggest providing two protein pools from each of a reference and an experimental sample. No where does Aebersold et al. indicate that the labeling pattern of the combined pools from the first experiment is compared to the labeling pattern of the combined pools from the second experiment (wherein the labeling was reversed), and that an inverse labeling pattern of a protein detected between the first and second experiments is indicative of a differentially expressed protein as recited in step (f) of amended independent Claim 1.

With respect to Chait et al., Applicants reiterate the arguments proffered to address the §102(e) rejection, namely that Chait et al. does not teach or specifically suggest comparing the labeling patterns obtained from the two experiments, nor does Chait et al. teach or specifically suggest that an inverse labeling pattern, a reversal in the signal intensity ratio of the isotopic pairs of a protein between the first experiment and the second experiment, is indicative of a differentially expressed protein as defined in step (f) of independent Claim 1. Thus, Aebersold et al. fail to remedy the deficiency present in Chait et al.

It is further noted that as acknowledged by the Examiner, the second experiment in Chait et al. was performed to ensure that any change in isotopic ratios was not caused by the isotopic enrichment itself (see, e.g., the outstanding Action page 8, lines 1-2). In contrast, one skilled in the art utilizing the chemical labeling as described in Aebersold et al., would not perform a second experiment, as was done in Chait et al., to confirm the isotopic ratios in the first experiment, since it would not be expected that isotopic labeling utilizing Aebersold's chemically identical protein labeling reagent would change the isotopic ratios. Accordingly, one skilled in the art armed with the knowledge of

Aebersold's chemical labeling method would not be motivated to utilize the Chait et al. method to perform a second experiment to confirm the results from the first experiment.

Accordingly, in view that 1) neither Aebersold et al. nor Chait et al. teach or specifically suggest comparing the labeling patterns obtained from two experiments as is set forth in step (f) of Claim 1, 2) nor do these references teach or specifically suggest that an inverse labeling pattern, a reversal in the signal intensity ratio of the isotopic pairs of a protein between the first experiment and the second experiment, is indicative of a differentially expressed protein, and 3) that one skilled in the art would not be motivated to combine Aebersold et al. with Chait et al., specifically Example 2, the combination of Aebersold et al. and Chait et al. does not make obvious Claims 13, 15 and 20 which depend from amended independent Claim 1.

In view of the above, withdrawal of the rejection of Claims 13, 15 and 20 under 35 U.S.C. §103(a) is respectfully requested.

Claims 29 and 31 have been rejected under 35 U.S.C. §103(a) as being unpatentable over Schnulzer et al., Chait et al. as applied to Claim 23 and further in view of Aebersold et al. In particular, the Examiner stated:

> "Schnolzer et al. and Chait et al. describe a method for identifying a differentially expressed protein as substantially described supra. The aforementioned references do not teach a fractionation step prior to step (a)."
>
> However, Aebersold et al. teach an affinity chromatographic fractionation of proteins (see e.g. col. 17, line 49, "panning") prior to step (a) as a preparative step in the analysis of membrane proteins (see col. 17, line 28). Therefore, it would have been obvious for a person of ordinary skill in the art to practice the method of identifying differentially expressed proteins, as taught by Schnolzer et at. and Chait et al., with a fractionation step prior to step (a) because Aebersold et al. discovered that panning and labeling membrane proteins with isotopic affinity tags can be used to identify important diagnostic or therapeutic targets (see col. 17, lines 49-52) without the step of solubilizing membrane proteins prior to analysis, thus avoiding a major complication facing prior art methods which require solubilization of membrane proteins prior to analysis."

Applicants traverse this rejection and respectfully submit that the combination of Schnolzer et al., Chait et al., and Aebersold et al. does not make obvious Claims 29 and 31.

The same arguments proffered above to address the §102(e) and §103(a) rejections apply equally to this rejection, namely that neither Schnolzer et al., Chait et al. or Aerbersold et al. each taken alone or combined teach or specifically suggest

comparing the labeling patterns obtained from the two experiments, or that an inverse labeling pattern of a peptide detected between the first and second experiments is indicative of a differentially expressed protein from which the peptide originated as set forth in step (f) of amended independent Claim 23. Further, as discussed above with respect to the rejection of Claims 13, 15 and 20, one skilled in art would not be motivated to combine Aebersold et al. with Chait et al. Accordingly, the combination of Schnolzer et al., Chait et al. and Aebersold et al. does not make obvious Claims 29 and 31 which depend from Claim 23.

In view of the above, withdrawal of the rejection of Claims 29 and 31 under 35 U.S.C. §103(a) is respectfully requested.

Claims 41-43 have been rejected under 35 U.S.C. §103(a) as being unpatentable over Schnolzer et al., Chait et al. as applied to Claim 35 and further in view of Aebersold et al. In making this rejection the Examiner reiterated the same remarks regarding Schnolzer et al., Chait et al. and Aebersold et al. as were made in the rejection of Claims 29 and 31 under 35 U.S.C. §103(a).

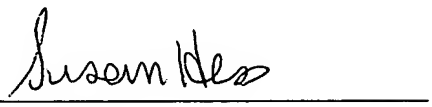
Applicants traverse this rejection and respectfully submit that the combination of Schnolzer et al., Chait et al. and Aebersold et al. does not make obvious Claims 41-43.

The same arguments proffered above to address the §102(e) and §103(a) rejections apply equally to the rejection of Claims 41-43, namely that neither Schnolzer et al., Chait et al. or Aerbersold et al. each taken alone or combined teach or specifically suggest comparing the labeling patterns obtained from the two experiments wherein the label was reversed in the two experiments, or that and that an inverse labeling pattern of a peptide detected between the first and second experiments is indicative of a differentially expressed protein from which the peptide originated as set forth in step (g) of amended independent Claim 35. Further, as discussed above with respect to the rejection of Claims 13, 15 and 20, one skilled in art would not be motivated to combine Aebersold et al. with Chait et al. Accordingly, the combination of Schnolzer et al., Chait et al. and Aebersold et al. does not make obvious Claims 41-43 which depend from Claim 35.

In view of the above, withdrawal of the rejection of Claims 41-43 under 35 U.S.C. §103(a) is respectfully requested.

A good faith effort has been made to place the present application in condition for allowance. If the Examiner believes a telephone conference would be of value, he is requested to call the undersigned at the number listed below.

Respectfully submitted,



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